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(54) **LIQUID ENZYME FORMULATION AND A PROCESS FOR ITS PREPARATION**

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(57) **ABSTRACT**

The present invention relates to a liquid enzyme formulation, particularly to a liquid and stable formulation comprising a crosslinking enzyme and/or an enzyme modifying milk proteins. Particularly the present invention relates a liquid and stable transglutaminase formulation. In addition, the present invention relates to a method for preparing a liquid enzyme formulation.

LIQUID ENZYME FORMULATION AND A PROCESS FOR ITS PREPARATION

FIELD OF THE INVENTION

[0001] The present invention relates to a liquid enzyme formulation, particularly to a liquid and stable formulation comprising a crosslinking enzyme and/or an enzyme modifying milk proteins. Particularly the present invention relates a liquid and stable transglutaminase formulation. In addition, the present invention relates to a method for preparing a liquid enzyme formulation.

BACKGROUND OF THE INVENTION

[0002] Enzyme formulations comprising a crosslinking enzyme and/or an enzyme modifying milk proteins, such as laccase, tyrosinase, peroxidase, sulfhydryl oxidase or glucose oxidase are commercially available both in powder and liquid formulations. However, transglutaminase and protein glutaminase products are currently in the market only in powder form. The use of a powdered enzyme product is not totally accepted due to the dust formation in all production plants. The health hazards resulting from the dusting have incurred concerns among the workers, especially.

[0003] A transglutaminase derived from *Streptoverticillium mobaraense* strain and a process for its preparation has been disclosed in the European patent No. 0 379 606 B1. Further, a method for the production of a transglutaminase using a gene isolated from *Streptomyces lydicus* strain is disclosed in the European Patent No. 0 777 726 B1.

[0004] One of the problems associated with formulating an enzyme, such as a transglutaminase, in liquid form, is the lack of stability of the formulation. Further, one of the disadvantages associated with the present liquid enzyme formulations is that they contain at least one preservative.

BRIEF DESCRIPTION OF THE INVENTION

[0005] An object of the present invention is thus to provide a liquid enzyme formulation comprising transglutaminase and/or another milk protein crosslinking and/or modifying enzyme such as laccase, tyrosinase, peroxidase, sulfhydryl oxidase, glucose oxidase or protein glutaminase, which is stable and can be stored for a time period required from a commercial formulation in a room temperature or in temperatures of a refrigerator and/or a freezer.

[0006] Another object of the present invention is to provide a liquid formulation comprising a transglutaminase, which is stable and can be stored for a time period required from a commercial formulation in a room temperature or in temperatures of a refrigerator and/or a freezer.

[0007] A further object of the present invention is to provide a method for the preparation of a stable, liquid enzyme formulation comprising transglutaminase and/or another milk protein crosslinking and/or modifying enzyme.

[0008] An even further object of the present invention is to provide a method for the preparation of a stable, liquid formulation comprising a transglutaminase.

[0009] The objects of the invention are achieved by the formulations and methods set forth in the independent claims. Preferred embodiments of the invention are described in the dependent claims.

[0010] Other objects, details and advantages of the present invention will become apparent from the following detailed description and examples.

DETAILED DESCRIPTION OF THE INVENTION

[0011] Milk protein crosslinking and/or modifying enzymes such as transglutaminase, laccase, tyrosinase, peroxidase, sulfhydryl oxidase and protein glutaminase catalyze milk protein modifications. There seems to be synergism within the action of these enzymes and further with the action of glucose oxidase. Without being bound by any theory glucose oxidase and/or peroxidase seem to catalyze reactions wherein oxygen is released through hydrogen peroxide formation. The oxygen can then catalyze (oxidate) crosslinking of tyrosinase.

[0012] Milk protein crosslinking and/or modifying enzymes such as transglutaminase, laccase, tyrosinase, peroxidase, sulfhydryl oxidase and protein glutaminase optionally together with glucose oxidase are used in the manufacture of processed fish, meat and egg products, pastes and pates, fruits, berries and vegetables, soy products, cereal products, bread and bakery products.

[0013] Following milk protein crosslinking and/or modifying enzymes are all relevant in food processing in dairy or other food categories.

[0014] Transglutaminases are a family of enzymes (EC 2.3.2.13) that catalyze the generation of covalent linkages between the glutamine and lysine amino acid residues present in the protein molecules. When linkages are formed, ammonia is released.

[0015] Laccases (EC 1.10.3.2) derived from fungi and bacteria, such as, fungus *Trametes hirsute*, catalyze the crosslinking between carbohydrates and proteins (oxidation of aromatic compounds and cysteine) with applications in food processing for reduction of allergenicity, for example.

[0016] Tyrosinases (EC 1.14.18.1) are enzymes which catalyzes the oxidation of phenols such as tyrosine, with applications in food processing for reduction of allergenicity, for example.

[0017] Peroxidases (EC 1.11.1.7) are a family of enzymes that catalyze the oxidation of aromatic compounds with applications in food processing for reduction of allergenicity, for example.

[0018] Sulfhydryl oxidase (EC 1.8.3.3) catalyzes the formation of disulfide bonds, oxidation of glutathione.

[0019] Protein glutaminase catalyzes the deamidation of protein bound glutamine, and glutamine is converted to glutamic acid.

[0020] Glucose oxidase catalyzes the formation of protein crosslinks and oxidative gelation of pentosans.

[0021] Milk protein crosslinking and/or modifying enzymes such as transglutaminase, laccase, tyrosinase, peroxidase, sulfhydryl oxidase and protein glutaminase are used in dairy industry to stabilize the structure of milk-based products. In addition to dairy industry, these enzymes are used in the manufacture of processed fish, meat and egg products, pastes and pates, fruits, berries and vegetables, soy products, cereal products, bread and bakery products. Accordingly, a liquid enzyme formulation is suited for the manufacture of dairy, processed fish, meat, egg, pastes and pates, fruits, berries and vegetables, soy, cereal, bread and bakery products, for example. The use of a liquid enzyme preparation would be more practical and advantageous than the use of powder formulation, especially in the industrial scale manufacturing.

[0022] Transglutaminases are most active in the pH range from 5.2 to 8. When a liquidized transglutaminase is stored at pH 5.2 or at pH 8, the enzyme loses its activity quickly. After

storing a transglutaminase at pH 5.2 for 7 days at room temperature or in the temperature of a refrigerator, only half of the activity is left.

[0023] The invention is based on the finding that when a transglutaminase, tyrosinase or protein glutaminase is stored in a suspension of a polyol, such as glycerol or sorbitol, and water in the pH range from 4.4 to 5.1, its activity is remained moderately during storage at room temperature and excellently during storage in the temperatures of a refrigerator and/or a freezer. In addition, the invention is based on the finding that when a transglutaminase together with a protein glutaminase is stored in a suspension of glycerol and water at pH 4.6, the activity of the enzymes remained moderately during storage at room temperature and excellently during storage in the temperatures of a refrigerator and/or a freezer. Further, the invention is based on the finding that when a transglutaminase together with a protein glutaminase and tyrosinase is stored in a suspension of glycerol and water at pH 4.6, the activity of the enzymes remained moderately during storage at room temperature and excellently during storage in the temperatures of a refrigerator and/or a freezer. In addition, the liquid preparations of the present invention are also microbiologically stable during the storage at room temperature and in the temperatures of a refrigerator and/or a freezer. Further, it was unexpectedly found that liquid enzyme formulation of the present invention maintained its enzyme activity and microbiological purity (no microbial growth) without any preservatives in the formulation in the temperatures of a refrigerator and/or a freezer.

[0024] In one embodiment, the liquid enzyme formulation in polyol-water suspension has pH value within the range from 4.4 to 5.1. In another embodiment of the present invention, the pH of the enzyme formulation is within the range from 4.4 to 4.8. In one embodiment of the present invention, the pH of the enzyme formulation is 4.4. In another embodiment of the present invention, the pH of the enzyme formulation is 4.6. In a further embodiment of the present invention, the pH of the enzyme formulation is 4.8. In an even further embodiment of the present invention, the pH of the enzyme formulation is 5.1.

[0025] Polyols, such as glycerol, sorbitol, xylitol and/or mannitol can be used in the liquid enzyme formulation of the present invention. Mixtures of the polyols, such as mixtures of glycerol and the other polyols are also operable.

[0026] The suspension of a polyol and water or a mixture of two or more polyols and water suitable for formulating the enzyme preparation of the present invention may contain the polyol(s), such as glycerol, sorbitol, xylitol and/or mannitol from 25% up to 100%, preferably from 50% up to 100% (w/w %). In one embodiment of the present invention, the enzyme is dissolved into 25% polyol/75% water (w/w) suspension. In another embodiment of the present invention, the enzyme is dissolved into 50% polyol/50% water (w/w) suspension. In a further embodiment of the present invention, the enzyme is dissolved into 75% polyol/25% water (w/w) suspension.

[0027] The suspension of glycerol and water suitable for formulating the enzyme preparation of the present invention may contain glycerol from 25% up to 100%, preferably from 50% up to 100%. In one embodiment of the present invention, the enzyme is dissolved into 25% glycerol/75% water suspension. In another embodiment of the present invention, the enzyme is dissolved into 50% glycerol/50% water suspension. In a further embodiment of the present invention, the enzyme is dissolved into 75% glycerol/25% water suspension. Further, the suspension of sorbitol and water suitable for formulating the enzyme preparation of the present invention may contain sorbitol from 25% up to 100%, preferably from 50% up to 100%. In one embodiment of the present invention, the enzyme is dissolved into 25% sorbitol/75% water suspension. In another embodiment of the present invention, the enzyme is dissolved into 50% sorbitol/50% water suspension. In a further embodiment of the present invention, the enzyme is dissolved into 75% sorbitol/25% water suspension.

[0028] The pH of the suspension may be adjusted to the desired range with an acid approved for food use, such as, lactic acid, GDL (glucono-deltalactone), citric acid, acetic acid, oxalic acid, malic acid, pantothenic acid, propionic acid and/or hydrochloric acid, or any mixtures/combinations thereof in the form of acid or salt. In one embodiment of the present invention, lactic acid is used for the pH adjustment.

[0029] The liquid enzyme preparation of the present invention may optionally contain also a preservative such as Na-benzoate. In one embodiment, the liquid enzyme preparation of the present invention does not contain any additional preservatives i.e., the formulation is free from preservatives. In another embodiment, the liquid enzyme preparation of the present invention contains an additional preservative. In a further embodiment, the liquid enzyme preparation of the present invention contains Na-benzoate as a preservative. In an even further embodiment, the liquid enzyme preparation of the present invention contains Na-benzoate, in an amount of 0.1 to 1%, preferably in an amount of 0.7% as a preservative.

[0030] The liquid enzyme preparation of the present invention maintains its activity in room temperature for about two weeks, in a refrigerator for about 1.5 to six months, at least and in freezer from a minimum of 5 months up to 24 months.

[0031] In one embodiment of the invention, the liquid formulation comprises one milk protein crosslinking and/or modifying enzyme. In another embodiment of the invention, the liquid formulation comprises two or more milk protein crosslinking and/or modifying enzymes. In one embodiment, the milk protein crosslinking and/or modifying enzyme in the formulation of the present invention is transglutaminase. In another embodiment, the milk protein cross-linking and/or modifying enzyme in the formulation of the present invention is tyrosinase. In another embodiment, the milk protein crosslinking and/or modifying enzyme in the formulation of the present invention is protein glutaminase. In another embodiment, the milk protein crosslinking and/or modifying enzymes in the formulation of the present invention are transglutaminase and protein glutaminase. In another embodiment, the milk protein crosslinking and/or modifying enzymes in the formulation of the present invention are transglutaminase and tyrosinase. In another embodiment, the milk protein crosslinking and/or modifying enzymes in the formulation of the present invention are transglutaminase and laccase. In a further embodiment, the milk protein crosslinking and/or modifying enzymes in the formulation of the present invention are transglutaminase, protein glutaminase and laccase. In an even further embodiment, the milk protein crosslinking and/or modifying enzymes in the formulation of the present invention are transglutaminase, protein glutaminase and tyrosinase.

[0032] The present invention relates also to a method for preparing a liquid enzyme formulation wherein a milk protein crosslinking and/or modifying enzyme is added to polyol-water suspension having pH value within the range from 4.4

to 5.1. In one embodiment of the present invention, the pH of the polyol-water suspension is adjusted to a value within the range from 4.4 to 5.1. In one embodiment of the present invention, the pH of the polyol-water suspension is adjusted to a value within the range from 4.4 to 4.8. In one embodiment of the present invention, the pH of the enzyme formulation is adjusted to pH 4.4. In another embodiment of the present invention, the pH of the polyol-water suspension is adjusted to pH 4.6. In a further embodiment of the present invention, the pH of the polyol-water suspension is adjusted to pH 4.8. In an even further embodiment of the present invention, the pH of the enzyme formulation is adjusted to pH 5.1.

[0033] The suspension of polyol and water suitable for the method of the present invention may contain polyol from 25% up to 100% (w-%). In one embodiment of the present invention, the enzyme is dissolved into 25% polyol-water suspension. In another embodiment of the present invention, the enzyme is dissolved into 50% polyol-water suspension. In a further embodiment of the present invention, the enzyme is dissolved into 75% polyol-water suspension. In one embodiment of the invention, the polyol is glycerol. In another embodiment of the invention, the polyol is sorbitol.

[0034] In the present method, the pH of the suspension may be adjusted to the desired range with an acid approved for food use (food grade, GRAS), such as, lactic acid, GDL, citric acid, acetic acid, oxalic acid, malic acid, pantothenic acid, propionic acid and/or hydrochloric acid or any mixtures/combinations thereof in the form of acid or salt. In one embodiment, lactic acid is used in the method of the present invention for the pH adjustment.

[0035] In the method of the present invention, also a preservative, such as Na-benzoate, may optionally be included in the liquid enzyme formulation. In one embodiment, the method of the present invention does not comprise addition of a preservative. In another embodiment, the method of the present invention comprises an addition of a preservative. In a further embodiment, the method of the present invention comprises addition of Na-benzoate as a preservative. In an even further embodiment, the method of the present invention comprises addition of Na-benzoate, in an amount of 0.1 to 1%, preferably in an amount of 0.7% as a preservative.

[0036] In one embodiment, the method of the present invention comprises the following steps:

[0037] a) pH of a polyol-water suspension is adjusted with food grade acid(s) to a value within the range from 4.4 to 5.1

[0038] b) a milk protein crosslinking and/or modifying enzyme is added to the suspension

[0039] c) optionally a preservative is added.

[0040] In another embodiment, the method of the present invention comprises the following steps:

[0041] a) a milk protein crosslinking and/or modifying enzyme is added to a polyol-water suspension

[0042] b) pH of the suspension is adjusted with food grade acid(s) to a value within the range from 4.4 to 5.1

[0043] c) optionally a preservative is added.

[0044] In one embodiment of the present invention, one milk protein crosslinking and/or modifying enzyme is added to the suspension. In another embodiment of the present invention, two or more milk protein crosslinking and/or modifying enzymes are added to the suspension. In one embodiment, the milk protein crosslinking and/or modifying enzyme is transglutaminase. In another embodiment, the milk protein crosslinking and/or modifying enzyme is tyrosinase. In another embodiment, the milk protein crosslinking and/or modifying enzyme is protein glutaminase. In another embodiment, the milk protein crosslinking and/or modifying enzymes are transglutaminase and protein glutaminase. In another embodiment, the milk protein crosslinking and/or modifying enzymes are transglutaminase and tyrosinase. In another embodiment, the milk protein crosslinking and/or modifying enzymes are transglutaminase and laccase. In a further embodiment, the milk protein crosslinking and/or modifying enzymes are transglutaminase, protein glutaminase and laccase. In an even further embodiment, the milk protein crosslinking and/or modifying enzymes are transglutaminase, protein glutaminase and tyrosinase.

[0045] The invention will be described in more detail by means of the following examples. The examples are not to be construed to limit the claims in any manner whatsoever.

EXAMPLES

Comparative Example 1

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspension at pH 5.2 at 4° C.

[0046] The transglutaminase derived from *Streptoverticillium mobaraense*-strain having activity of 16.300 nkat/g (Activa® TG, Ajinomoto), was dissolved in activity of 274 nkat/g into 50% glycerol-water (w/w) suspension which pH was adjusted to 5.2 with lactic acid. The enzyme activity was monitored for 7 days. Additionally, the microbiological purity of the preparation was monitored.

[0047] On day 7, only 50% of the activity of the transglutaminase was left. No microbial growth was detected during the seven day preservation test.

Comparative Example 2

Transglutaminase Preparation Activa® TG (Ajinomoto) in Water at pH 5.2 at 4° C.

[0048] The transglutaminase Activa® TG (Ajinomoto) was dissolved in activity of 274 nkat/g into water having pH 5.2 adjusted with lactic acid. The suspension contained also 0.7% Na-benzoate as a preservative.

[0049] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0050] On day 50, only 43% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 1

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0051] The transglutaminase Activa® TG (Ajinomoto) was dissolved in activity of 274 nkat/g into 50% glycerol-water (w/w) suspension having pH 4.6 adjusted with lactic acid.

[0052] The enzyme activity was monitored for 7 days. Additionally, the microbiological purity of the preparation was monitored.

[0053] On day 7, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the seven day preservation test.

Example 2

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0054] The transglutaminase Activa® TG (Ajinomoto) was dissolved in activity of 326 nkat/g into 50% glycerol-water (w/w) suspension having pH 4.6 adjusted with lactic acid. The suspension contained also 0.7% Na-benzoate as a preservative.

[0055] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0056] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 3

Transglutaminase Preparation Activa® TG-YG (Ajinomoto) in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0057] The liquid transglutaminase formulation was prepared by dissolving Ajinomoto's transglutaminase preparation Activa® TG-YG, which contains a transglutaminase derived from *Streptoverticillium mobaraense*-strain and glutathione into 50% glycerol-water (w/w) suspension having pH 4.6 adjusted with lactic acid.

[0058] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0059] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 4

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspension at pH 4.4 at 4° C.

[0060] The liquid transglutaminase preparation was prepare by dissolving transglutaminase Activa® TG (Ajinomoto) in activity of 274 nkat/g into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.4.

[0061] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0062] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 5

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspension at pH 4.8 at 4° C.

[0063] The liquid transglutaminase preparation was prepare by dissolving transglutaminase Activa® TG (Ajinomoto) in activity of 274 nkat/g into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.8.

[0064] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0065] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 6

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspension at pH 5.1 at 4° C.

[0066] The liquid transglutaminase preparation was prepare by dissolving transglutaminase Activa® TG (Ajinomoto) in activity of 274 nkat/g into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 5.1 at 4° C.

[0067] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0068] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 7

Transglutaminase Preparation Saprona TG (Yiming Biological Products Co, China) in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0069] The liquid transglutaminase preparation was prepare by dissolving transglutaminase Yiming Saprona TG derived from *Streptoverticillium mobaraense*-strain into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.6.

[0070] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0071] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 8

Transglutaminase Preparation Reactyn CL 1000 TG (Campus SpA, Italy) in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0072] The liquid transglutaminase preparation was prepare by dissolving transglutaminase Campus Reactyn CL 1000 TG into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.6.

[0073] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0074] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 9

Transglutaminase Preparation TG-PG (Ajinomoto) in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0075] The liquid preparation was prepare by dissolving into 50% glycerol-water (w/w) suspension having pH 4.6 adjusted with lactic acid, an enzyme preparation TG-PG (Ajinomoto). The preparation TG-PG (Ajinomoto) contains a transglutaminase derived from *Streptoverticillium mobaraense*-strain and a protein glutaminase derived from *Chryseobacterium proteolyticum.*

[0076] The transglutaminase activity of the liquid preparation was 100 U/g and the protein glutaminase activity of the liquid preparation was 100 U/g.

[0077] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0078] On day 50, 100% of the activity of the transglutaminase and 100% of the activity of protein glutaminase were left. No microbial growth was detected during the 50 day preservation test.

Example 10

Transglutaminase Preparation Activa® TG (Ajinomoto) in 75% Glycerol/25% Water Suspension at pH 4.6 at 4° C.

[0079] The transglutaminase Activa® TG (Ajinomoto) was dissolved in activity of 326 nkat/g into 75% glycerol/25% water (w/w) suspension having pH 4.6 adjusted with lactic acid.

[0080] The enzyme activity was monitored for 50 days at the temperature of 4° C. Additionally, the microbiological purity of the preparation was monitored.

[0081] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the preservation test.

Example 11

Transglutaminase Preparation Activa® TG (Ajinomoto) in 25% Glycerol/75% Water Suspension at pH 4.6 at 4° C.

[0082] The transglutaminase Activa® TG (Ajinomoto) was dissolved in activity of 326 nkat/g into 25% glycerol/75% water (w/w) suspension having pH 4.6 adjusted with lactic acid.

[0083] The enzyme activity was monitored for 50 days at the temperature of 4° C. Additionally, the microbiological purity of the preparation was monitored.

[0084] On day 50, 72% of the activity of the transglutaminase was left. No microbial growth was detected during the preservation test.

Example 12

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspensions at pH 4.4-4.8 at 22° C.

[0085] The liquid transglutaminase preparations were prepare by dissolving transglutaminase Activa® TG (Ajinomoto) in activity of 2789 nkat/g into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.4-4.8.

[0086] The enzyme activity of the preparations was monitored for 13 weeks at the temperature of 22° C. Additionally, the microbiological purity of the preparations was monitored.

[0087] After two weeks storage, the activity of the enzyme in the preparations started decreasing. No microbial growth was detected during the preservation test.

Example 13

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspensions at pH 4.4-4.8 at 4° C.

[0088] The liquid transglutaminase preparations were prepare by dissolving transglutaminase Activa® TG (Ajinomoto) in activity of 2789 nkat/g into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.4-4.8.

[0089] The enzyme activity of the preparations was monitored for 26 weeks at the temperature of 4° C. Additionally, the microbiological purity of the preparations was monitored.

[0090] After 26 weeks storage, 89% of the activity of the enzyme was left. No microbial growth was detected during the preservation test.

Example 14

Transglutaminase Preparation Activa® TG (Ajinomoto) in Glycerol-Water Suspensions at pH 4.4-4.8 at −20° C.

[0091] The liquid transglutaminase preparations were prepare by dissolving transglutaminase Activa® TG (Ajinomoto) in activity of 2789 nkat/g into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.4-4.8.

[0092] The enzyme activity of the preparations was monitored for 26 weeks at the temperature of −20° C. Additionally, the microbiological purity of the preparations was monitored.

[0093] After 26 weeks storage, 97% of the activity of the enzyme was left. No microbial growth was detected during the preservation test.

Example 15

Tyrosinase Preparation in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0094] The tyrosinase enzyme was dissolved in activity of 100 U/g into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.6.

[0095] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0096] After 50 days storage, 97% of the activity of the tyrosinase was left. No microbial growth was detected during the preservation test.

Example 16

Protein Glutaminase Preparation in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0097] The protein glutaminase was dissolved in activity of 100 U/g into 50% glycerol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.6.

[0098] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0099] After 50 days storage, 96% of the activity of the protein glutaminase was left. No microbial growth was detected during the preservation test.

Example 17

Transglutaminase Preparation Activa® TG (Ajinomoto) in Sorbitol-Water Suspension at pH 4.6 at 4° C.

[0100] The liquid transglutaminase preparation was prepared by dissolving transglutaminase Activa® TG (Ajinomoto) in activity of 274 nkat/g into 50% sorbitol-water (w/w) suspension which pH was adjusted with lactic acid to pH 4.6.

[0101] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0102] On day 50, 100% of the activity of the transglutaminase was left. No microbial growth was detected during the 50 day preservation test.

Example 18

Liquid Enzyme Preparation Containing TG-PG (Ajinomoto) and Tyrosinase in Glycerol-Water Suspension at pH 4.6 at 4° C.

[0103] The liquid preparation was prepare by dissolving into 50% glycerol-water (w/w) suspension having pH 4.6 adjusted with lactic acid, an enzyme preparation TG-PG (Ajinomoto) and tyrosinase. The transglutaminase activity of the liquid preparation was 100 U/g, the protein glutaminase activity of the liquid preparation was 100 U/g and tyrosinase activity of the liquid preparation was 100 U/g.

[0104] The enzyme activity was monitored for 50 days. Additionally, the microbiological purity of the preparation was monitored.

[0105] On day 50, 100% of the activity of the transglutaminase, 100% of the activity of protein glutaminase and 98% of the activity of tyrosinase were left. No microbial growth was detected during preservation test.

[0106] It will be obvious to a person skilled in the art that, as the technology advances, the inventive concept can be implemented in various ways. The invention and its embodiments are not limited to the examples described above but may vary within the scope of the claims.

1. A liquid enzyme formulation comprising at least one milk protein crosslinking and/or modifying enzyme in polyol-water suspension comprising from 25% to 100% (w/w) polyol and having pH value within the range from 4.4 to 5.1.

2. The formulation according to claim **1**, wherein the polyol-water suspension comprises from 50% to 75% polyol.

3. The formulation according to claim **1**, wherein the polyol is glycerol or sorbitol.

4. The formulation according to claim **1**, wherein the pH is 4.6.

5. The formulation according to claim **1**, wherein the formulation comprises transglutaminase, tyrosinase or protein glutaminase.

6. The formulation according to claim **1**, wherein the formulation comprises transglutaminase and protein glutaminase.

7. The formulation according to claim **5**, wherein the formulation comprises also laccase and/or tyrosinase.

8. The formulation according to claim **1**, wherein the formulation is free from preservatives.

9. A method for preparing a liquid enzyme formulation, wherein at least one milk protein crosslinking and/or modifying enzyme is added to polyol-water suspension comprising from 25% to 100% (w/w) polyol and having pH value within the range from 4.4 to 5.1.

10. The method according to claim **9**, wherein the method comprises the following steps:

a) pH of the polyol-water suspension is adjusted with food grade acid to a value within the range from 4.4 to 5.1,

b) at least one milk protein crosslinking and/or modifying enzyme is added to the suspension,

c) optionally a preservative is added.

11. The method according to claim **9**, wherein the method comprises the following steps:

a) at least one milk protein crosslinking and/or modifying enzyme is added to a polyol-water suspension comprising from 25% to 100% polyol,

b) pH of the suspension is adjusted with food grade acid(s) to a value within the range from 4.4 to 5.1

c) optionally a preservative is added.

12. The method according to claim **9**, wherein the polyol-water suspension comprises from 50% to 75% polyol.

13. The method according to claim **9**, wherein the pH of the polyol-water suspension is 4.6.

14. The method according to claim **9**, wherein the polyol is glycerol or sorbitol.

15. The method according to claim **9**, wherein the enzyme is transglutaminase, tyrosinase or protein glutaminase.

16. The method according to claim **9**, wherein the enzymes are transglutaminase and protein glutaminase.

17. The method according to claim **15**, wherein also laccase and/or tyrosinase is added to the suspension.

18. The method according to claim **9**, wherein the method does not comprise addition of a preservative.

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